molecular probes
life technologies

WesternDot® Secondary Antibody Conjugates

with VIVID® Technology

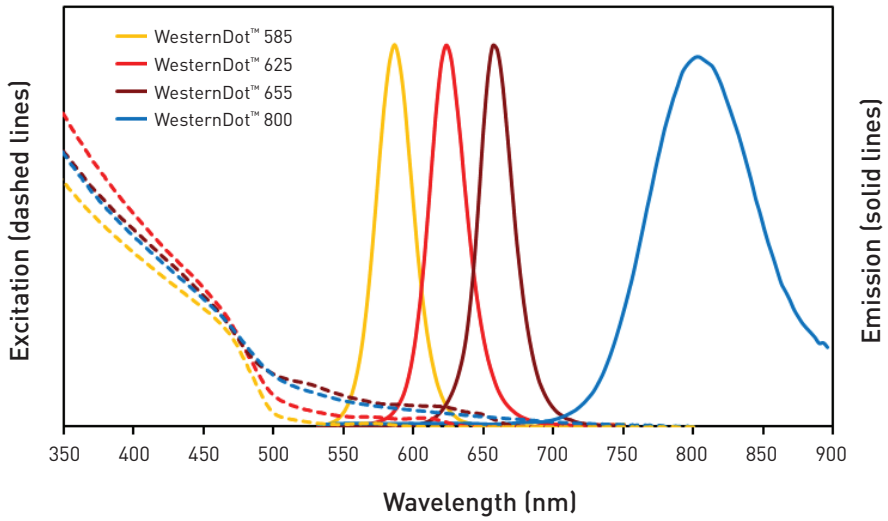
Table 1 Contents and storage

Material	Amount	Pack size	Storage	Stability
WesternDot® Secondary Antibody Conjugates	500 µL	25 blots	• 2–8°C • DO NOT FREEZE	When stored as directed, the product is stable for at least 6 months.
Approximate absorption and emission maxima: See Figure 1, below.				

Introduction

WesternDot® reagents allow detection of proteins that have been immobilized on membranes (nitrocellulose or PVDF) following western transfer. Detection is accomplished with WesternDot® secondary-antibody conjugates that utilize Qdot® nanocrystals enhanced with VIVID® technology, making them brighter than the original Qdot® reagents. The unique fluorescent properties of WesternDot® reagents allow simultaneous detection of multiple proteins on a single blot without stripping and reprobing. The stability of the WesternDot® reagents makes it possible to take multiple images and store dried blots for months with minimal loss of fluorescent signal. Protein bands can be captured with fluorescent imagers equipped with UV, violet, or deep-blue excitation wavelengths, including gel imagers with a UV transilluminator.

Figure 1 Typical absorption and emission spectra of WesternDot® Secondary Antibody Conjugates.



For Research Use Only. Not for use in diagnostic procedures.

Before You Begin

Materials required but not provided

- Primary antibody to detect applied antigen
- Purified water, 1× PBS, or 1× TBS
- Casein or non-fat powdered milk for blocking
- UV transilluminator/ethidium bromide filter (for WesternDot® 625 conjugate) or fluorescence imaging system (for WesternDot® 585, 655, and 800 conjugates). See Table 2, page 4, for a partial list of compatible instruments.

Disposal of WesternDot® conjugates

Dispose of the material in compliance with all applicable local, state, and federal regulations. For more information on the composition of these materials, consult the Safety Data Sheets (SDSs), which are available at **www.lifetechnologies.com/sds**. Dispose of this material as hazardous waste.

Guidelines for using WesternDot® conjugates

- While WesternDot® conjugates are compatible with both PVDF and nitrocellulose membranes, we recommend nitrocellulose membranes for best results.
- Use common buffers such as phosphate-buffered saline (1× PBS) or tris-buffered saline (1× TBS) to prepare blocking, incubation, and washing solutions.
- Avoid using Tween® 20-containing buffers (e.g. TBST) as they may cause high/uneven background, particularly when using PVDF membranes.
- For washing, use 1× buffer (PBS or TBS) or purified water.
- Use 2% (w/v) casein (sodium salt) in PBS or TBS buffer for all blocking and incubation solutions for best results. Casein (sodium salt) is the soluble form of a protein derived from milk and is available from several suppliers, such as Sigma-Aldrich or Thermo Fisher Scientific. Solutions of 5% (w/v) non-fat powdered milk may be substituted for casein solutions.
- Do not use bovine serum albumin (BSA)-containing solutions for blocking or incubating WesternDot® conjugates as their use may cause high background and/or reduced signal.
- For primary antibodies that are incompatible with casein or milk (e.g., many anti-phosphoprotein antibodies), use a 0.5% BSA-containing solution for primary antibody incubation only (Step 3, page 3). In all other steps, 2% casein or 5% non-fat milk solution should be used.
- We recommend using a 1:500 dilution of the WesternDot® conjugate. Further optimization may be necessary for best results.
- WesternDot® reagents work well when excited by any light source with a wavelength shorter than their emission. However, best results are obtained when they are excited in the UV, violet, or deep-blue light. When utilizing a transillumination light source, place the blots **face down** (protein-side down) for optimal absorption of the excitation source. Transillumination of nitrocellulose with UV light may result in a slight yellowing of the membrane; however, this does not affect product performance.

Western-Blot staining with WesternDot® conjugates

1. Prepare the blocking/incubation solution by dissolving 1 g of casein (sodium salt) in 50 mL of 1× PBS or 1× TBS. See **Guidelines for Using WesternDot® Conjugates**, page 2, for more information on blocking and incubation buffers.

2. Block the membrane with 10 mL blocking/incubation solution for one hour at room temperature on an orbital shaker.

3. Prepare primary antibody solution by diluting antibody solution to the manufacturer's recommended concentration (typically ~1 µg/mL) in 10 mL blocking/incubation solution.

4. After decanting the blocking/incubation solution, incubate the membrane in the primary antibody solution. For best signal, we recommend overnight incubation at 4 ºC on an orbital shaker. If a shorter incubation time is required, incubate for 1–3 hours at room temperature on an orbital shaker.

5. After decanting the primary antibody solution,wash the membrane 3 times, 5 minutes each, with 20 mL of 1× PBS (or 1× TBS).

6. Prepare the WesternDot® incubation solution by adding 20 µL of the WesternDot® conjugate to 10 mL blocking/incubation solution (1:500 dilution).

7. Incubate the membrane in the WesternDot® incubation solution for 60 minutes at room temperature on an orbital shaker.

8. Decant the WesternDot® incubation solution and wash the membrane 3 times, 5 minutes each, with 20 mL of 1× PBS (or 1×TBS).

9. Rinse the membrane 2 times, 30 second each, with 20 mL purified water. These rinses remove salt from the membrane to improve image quality and archivability of dried membranes.

10. Air-dry the membrane. Hanging the membrane (with a paper clip, alligator clip, etc.) helps ensure even drying.

11. Image the membrane.

    Note: When imaging blots with a transilluminator, the blot **must** be placed **protein-side down** on the light source. Otherwise, the signal will be limited.

Multiple antigen staining

When detecting multiple antigens, use primary antibodies from different host species (for example, mouse and rabbit). This avoids cross-reactivity between primary antibodies and prevents the WesternDot® secondary from detecting the wrong primary antibody. You may combine primary antibodies from different host species into a single staining solution (Step 3) for simultaneous incubation (Step 4).

To detect the primary antibodies, use a different WesternDot® secondary for each primary. When selecting secondary antibodies, you must consider the intra-species interactions. Avoid picking WesternDot® secondaries from the same species to prevent them from binding to each other rather than their respective primary antibodies. For example, do not use a WesternDot® donkey anti-goat antibody conjugate if the other secondary you are using is a goat anti-rabbit conjugate. You may combine different WesternDot® secondary antibodies into a single staining solution (Step 6) for simultaneous incubation (Step 7).

If you know your target's size, and there is separation between them on the blot, you may utilize WesternDot® secondaries that have some overlap in their emission.

Table 2 Imaging platforms recommended for detecting WesternDot® Secondary Antibody conjugates. For basic gel imagers, use WesternDot® 625 with EtBr filters. Some manufacturers offer alternate filter options (e.g. SYBR® Green; SYBR® Gold) that may be compatible with other WesternDot® colors. Refer to the specifications of your instrument for additional filter information.

Manufacturer	Platform	Compatible WesternDot® reagents	Standard filters for detecting WesternDot® reagents*
Life Technologies	E-Gel® Imager	585, 625	WesternDot® 585: Orange WesternDot® 625: Orange
	Safe Imager™	625	WesternDot® 625: EtBr
GE Healthcare	ImageQuant LAS 4000 & 4010	585, 625, 655, 800	WesternDot® 625: EtBr
	Typhoon FLA 9000	585, 625, 655, 800	WesternDot® 585: 526SP, 560LP, 610BP30 WesternDot® 625: 520BP40, 555BP20, 580BP30, 610BP30 WesternDot® 655: 526SP, 560LP, 670BP30 WesternDot® 800: 526SP, 560LP
Protein Simple	FluorChem M	585, 625, 655, 800	WesternDot® 585: 593BP40, 607BP36 WesternDot® 625: 593BP40, 607BP36 WesternDot® 655: 607BP36, 710BP40 WesternDot® 800: 710BP40
	FluorChem E	585, 625, 655	WesternDot® 585: 537BP35, 590BP50, 620BP40 WesternDot® 625: 590BP50, 620BP40 WesternDot® 655: 620BP40
	Fluorchem FC3	585, 625, 655	WesternDot® 585: 595BP55 WesternDot® 625: 595BP55 WesternDot® 655: 595BP55
	FluorChem HD2		
	FluorChem Q	585, 625, 655, 800	WesternDot® 585: 537BP35, 595BP55, 606BP62 WesternDot® 625: 537BP35, 595BP55, 606BP62 WesternDot® 655: 537BP35, 595BP55, 606BP62, 699BP62 WesternDot® 800: 699BP62
	FluorChem R	585, 625, 655, 800	WesternDot® 585: 537BP26, 593BP40, 607BP36 WesternDot® 625: 593BP40, 607BP36 WesternDot® 655: 607BP36, 710BP40 WesternDot® 800: 710BP40, 835BP70
	AlphaImager HP, Red, Mini	585, 625, 655	WesternDot® 585: 595BP55 WesternDot® 625: 595BP55 WesternDot® 655: 595BP55
SYNGENE	G:BOX Chemi XR5	585, 625, 655, 800	
	G:BOX Chemi XL1.4		
	G:BOX Chemi XT4		
	G:BOX Chemi XX8		
	G:BOX Chemi IR6		
Pierce	myECL Imager	585, 625, 655	WesternDot® 585: 592BP27 WesternDot® 625: 592BP27

WesternDot® products work well when excited by any light source with a wavelength shorter than their emission. However, best results are obtained when they are excited with UV, violet, or deep-blue light.

* The filters listed are standard filters that are provided with the instrument, other filters may also be suitable.

Table 2 Imaging platforms recommended for detecting WesternDot® Secondary Antibody conjugates, continued.

Manufacturer	Platform	Compatible WesternDot® reagents	Standard filters for detecting WesternDot® reagents*
Bio-Rad	ChemiDoc MP System	585, 625, 655, 800	WesternDot® 585: 535-640 WesternDot® 625: 535-640 WesternDot® 655: 535-640
	ChemiDoc XRS+ System	585, 625, 655	
	Gel Doc XR+ System	585, 625, 655	
	PharosFX	585, 625, 655	WesternDot® 625: 640BP40 WesternDot® 655: 640BP40
	PharosFX Plus	585, 625, 655, 800	WesternDot® 585: 390LP WesternDot® 625: 390LP, 640BP40 WesternDot® 655: 390LP, 640BP40
	VersaDoc MP System	585, 625, 655, 800	WesternDot® 585: 530BP28, 605BP50 WesternDot® 625: 605BP50 WesternDot® 655: 605BP50
UVP	UV Transilluminators	625	WesternDot® 625: various
	BioSpectrum 500, 600	585, 625, 655, 800	WesternDot® 585: 515-570, 485-655, 570-640 WesternDot® 625: 485-655, 570-640 WesternDot® 655: 485-655, 570-640
	BioDoc-It 210, 220	585, 625, 655	WesternDot® 585: 605BP35 WesternDot® 625: 605BP35
DNR	MiniBIS Pro	585, 625, 655	WesternDot® 625: Orange
Kodak	Gel Logic	585, 625, 655	WesternDot® 585: 590 WesternDot® 625: 590
	Image Station 4000 MM and PRO	585, 625, 655, 800	WesternDot® 585: 440-830 WesternDot® 625: 440-830 WesternDot® 655: 440-830 WesternDot® 800: 440-830
	Image Station 4000R and PRO	585, 625, 655	WesternDot® 585: 435-670 WesternDot® 625: 435-670 WesternDot® 655: 435-670
Alpha Innotech	AlphaDigiDoc PRO	585, 625, 655	
	AlphaImager® EP		
	AlphaImager® HP		
LiCOR	ODYSSEY CLx	800	WesternDot® 800: 700BP, 800BP
	ODYSSEY Sa		
	ODYSSEY Fc	655, 800	

WesternDot® products work well when excited by any light source with a wavelength shorter than their emission. However, best results are obtained when they are excited with UV, violet, or deep-blue light.

* The filters listed are standard filters that are provided with the instrument, other filters may also be suitable.

Troubleshooting

Problem	Possible cause	Possible solution
Weak or no signal	Poor or incomplete transfer	Make sure transfer apparatus and membrane sandwiches are assembled correctly. Use appropriate transfer times. Repeat blot. After blotting, stain membrane to measure transfer efficiency.
	Protein of interest ran off the gel	Use positive control and/or molecular weight marker to match gel separation range to size of protein being blotted. After blotting, stain membrane to measure transfer efficiency.
	Membrane not completely wetted	Follow instructions for pre-wetting the membrane.
	Inactive or overly dilute primary antibody	Determine antibody activity by performing a dot blot. Increase antibody concentration as necessary.
	Sample too dilute	Load a larger amount of protein onto the gel.
	Blot incorrectly imaged	Blot must be placed protein-side towards the light source (i.e., face down on a transilluminator or face up on an epifluorecent imager).
	Blots are too old	Protein may have degraded over time. Use freshly prepared blots
	Primary antibody is not compatible with incubation buffer	Some antibodies (e.g. anti-phosphoproteins and anti-actin) are not compatible with casein or milk. We recommend using 0.5% BSA or even no carrier protein in the primary antibody incubation buffer. If you are still having difficulties, try 1x fish serum (e.g. Seablock) in the blocking and WesternDot® incubation steps.
	Excitation or emission filters not compatible with reagents	Check instrument specifications to be sure there is a good match with the absorption and emission spectra of the regents.
High background	Blocking time or washing time is too short	Perform each step for the specified amount of time.
	Primary and/or secondary antibody concentration too high	Determine optimal antibody concentration by performing a dot blot. Decrease antibody concentration as necessary.
	Membrane not completely wetted	The incubation dish must be small enough to allow thorough coverage of membrane by blocking/incubation buffer to prevent drying. Shake or agitate during each step.
	Incompatible buffer formulations	Perform each step in a buffer specified in the protocol. Avoid buffers containing Tween®-20, especially on PVDF membranes. Avoid using BSA as a component in the blocking or WesternDot® reagent incubation buffers.
	Short blocking time or long washing time	Make sure that each step is performed for the specified amount of time.
Uneven, patterned, or spotted background	Membrane, solutions, or incubation tray is contaminated	Use clean glassware and purified water to prepare solutions. Replace or clean the tray thoroughly with a glassware-cleaning detergent. Rinse thoroughly with purified water. Wear clean gloves at all times. Use forceps when handling membranes.
	Membrane is contaminated by fingerprints	Wear clean gloves at all times and use forceps when handling membranes. Always handle membranes around the edges.
	Membrane blotting pads are dirty or contaminated	Soak pads with detergent and rinse thoroughly with purified water before use. Replace pads when they become worn or discolored.
	Membrane is dried on a patterned paper towel	Dry the membrane by hanging (e.g. using a paper clip).
	Blocking was uneven.	The incubation dish must be sufficient to allow thorough coverage of membrane. Shake or agitate during each step.
Saturated signal	Protein is overloaded	Reduce load or dilute concentration of sample.
	Exposure time is too long	Reduce exposure time.
Uneven protein bands	Protein ran too fast	Slow the protein migration rate in the gel by using lower electrophoresis voltage and/or current.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product name	Unit size
W10800	WesternDot® 585 Donkey Anti-Mouse *with VIVID® technology*	500 µL
W10801	WesternDot® 585 Donkey Anti-Rabbit *with VIVID® technology*	500 µL
W10802	WesternDot® 585 Donkey Anti-Goat *with VIVID® technology*	500 µL
W10803	WesternDot® 585 Goat Anti-Mouse *with VIVID® technology*	500 µL
W10804	WesternDot® 585 Goat Anti-Rabbit *with VIVID® technology*	500 µL
W10805	WesternDot® 625 Donkey Anti-Mouse *with VIVID® technology*	500 µL
W10806	WesternDot® 625 Donkey Anti-Rabbit *with VIVID® technology*	500 µL
W10807	WesternDot® 625 Donkey Anti-Goat *with VIVID® technology*	500 µL
W10808	WesternDot® 625 Goat Anti-Mouse *with VIVID® technology*	500 µL
W10809	WesternDot® 625 Goat Anti-Rabbit *with VIVID® technology*	500 µL
W10810	WesternDot® 655 Donkey Anti-Mouse *with VIVID® technology*	500 µL
W10811	WesternDot® 655 Donkey Anti-Rabbit *with VIVID® technology*	500 µL
W10812	WesternDot® 655 Donkey Anti-Goat *with VIVID® technology*	500 µL
W10813	WesternDot® 655 Goat Anti-Mouse *with VIVID® technology*	500 µL
W10814	WesternDot® 655 Goat Anti-Rabbit *with VIVID® technology*	500 µL
W10815	WesternDot® 800 Goat Anti-Mouse *with VIVID® technology*	500 µL
W10816	WesternDot® 800 Goat Anti-Rabbit *with VIVID® technology*	500 µL
W10819	WesternDot® 655 Goat Anti-Rat *with VIVID® technology*	500 µL
W10820	WesternDot® 655 Goat Anti-Chicken *with VIVID® technology*	500 µL
W10821	WesternDot® 800 Goat Anti-Rat *with VIVID® technology*	500 µL
W10822	WesternDot® 800 Goat Anti-Chicken *with VIVID® technology*	500 µL
W10823	WesternDot® 800 Donkey Anti-Mouse *with VIVID® technology*	500 µL
W10824	WesternDot® 800 Donkey Anti-Rabbit *with VIVID® technology*	500 µL
W10825	WesternDot® 800 Donkey Anti-Goat *with VIVID® technology*	500 µL

Purchaser Notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/sds.

Certificate of Analysis

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14 August 2014

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